

REMARKS

Claims 1-30 are presently pending. By amendment herein, one of the selected phenotypes recited in claim 23 has been removed from claim 23 and added in new claim 30. Support for these amendments can be found throughout the specification as filed. No new matter has been added as a result of these amendments and entry thereof is respectfully requested prior to substantive examination.

Respectfully submitted,

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Version Showing Changes Made to Claims

23. (Amended) The method of claim 1, wherein the selected phenotype is related to cancer, nephritis, prostate hypertrophy, hematopoiesis, osteoporosis, obesity, [cardiovascular disease,] or diabetes.

30. (New) The method of claim 1, wherein the selected phenotype is related to cardiovascular disease.



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WHAT IS CLAIMED IS

1. A method of identifying a gene or genes associated with a selected phenotype, the method comprising the steps of:
 - (a) providing a nucleic acid library comprising nucleotide sequences that encode at least partially randomized zinc finger proteins;
 - (b) transducing cells with expression vectors, each comprising a nucleotide sequence from the library;
 - (c) culturing the cells so that zinc finger proteins are expressed in the cells, wherein the zinc finger proteins modulate gene expression in at least some of the cells;
 - (d) assaying the cells for a selected phenotype and determining whether or not the cells exhibit the selected phenotype; and
 - (e) identifying, in cells that exhibit the selected phenotype, the gene or genes whose expression is modulated by expression of a zinc finger protein, wherein the gene so identified is associated with the selected phenotype.
2. The method of claim 1, wherein the zinc finger protein has three, four, or five fingers.
3. The method of claim 1, wherein the library comprises no more than 10^7 clones.
4. The method of claim 1, wherein the cells are physically separated, individual pools of cells and each individual pool of cells is transduced with an expression vector comprising a nucleotide sequence from the library.
5. The method of claim 4, wherein the physical separation of the pools of cells is accomplished by placing each pool of cells in a separate well of a 96, 384, or 1536 well plate.
6. The method of claim 4, wherein the cells are assayed for the selected phenotype using liquid handling robots.
7. The method of claim 1, wherein the cells are pooled together and transduced in a batch.
8. The method of claim 7, wherein the cells are assayed for the selected phenotype using flow cytometry.
9. The method of claim 1, wherein the library is made by finger grafting, DNA shuffling, or codon doping.
10. The method of claim 1, wherein the zinc finger proteins are fusion proteins comprising a regulatory domain.
11. The method of claim 10, wherein the zinc finger proteins are fusion proteins comprising at least two regulatory domains.
12. The method of claim 10, wherein the regulatory domain is selected from the group

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consisting of a transcriptional repressor, a methyl transferase, a transcriptional activator, a histone acetyltransferase, and a histone deacetylase.

13. The method of claim 10, wherein the regulatory domain is VP16 or KRAB.
14. The method of claim 1, wherein modulation of gene expression is repression of gene expression.
15. The method of claim 1, wherein modulation of gene expression is activation of gene expression.
16. The method of claim 1, wherein the cells are selected from the group consisting of animal cells, plant cells, bacterial cells, protozoal cells, or fungal cells.
17. The method of claim 1, wherein the cells are mammalian cells.
18. The method of claim 1, wherein the cells are human cells.
19. The method of claim 1, wherein expression of the zinc finger proteins is controlled by administration of a small molecule.
20. The method of claim 19, wherein the small molecule is tetracycline.
21. The method of claim 1, wherein the expression vectors are a viral vector.
22. The method of claim 21, wherein the expression vectors are a retroviral expression vector, a lentiviral expression vector, an adenoviral expression vector, or an AAV expression vector.
23. (Amended) The method of claim 1, wherein the selected phenotype is related to cancer, nephritis, prostate hypertrophy, hematopoiesis, osteoporosis, obesity, or diabetes.
24. The method of claim 1, wherein the zinc finger proteins comprise a Zif268 backbone.
25. The method of claim 1, wherein genes that are associated with the selected phenotype are identified by comparing differential gene expression patterns in the presence and absence of expression of the zinc finger protein.
26. The method of claim 25, wherein differential gene expression patterns are compared using an oligonucleotide array.
27. The method of claim 1, wherein genes that are associated with the selected phenotype are identified by using zinc finger proteins from the library of randomized zinc finger proteins to probe YAC or BAC clones.
28. The method of claim 1, wherein genes that are associated with the selected

phenotype are identified by scanning genomic sequences for target sequences recognized by zinc finger proteins from the library of randomized zinc finger proteins.

29. The method of claim 1, wherein genes that are associated with the selected phenotype are identified by cross-linking the zinc finger protein to DNA with which it is associated, followed by immunoprecipitation of the zinc finger protein and sequencing of the DNA.

30. (New) The method of claim 1, wherein the selected phenotype is related to cardiovascular disease.